(12) AUSTRALIAN PATENT ABSTRACT
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(54) LIPIDS AND TENSIDS IN AQUEOUS PHASE

(71) CIBA-GEIGY AG
(21) 17402/83 (22) 28.7.83 (24) 29.7.82
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(57)

There is great interest in the therapeutic use of liposomes as carriers for a very wide range of active ingredients. Accordingly, liposomes have been proposed as carriers for proteins, e.g. antibodies or enzymes, hormones, vitamins or genes or, for analytical purposes, as carriers for marker compounds. For example, US patent 3 933 754 describes a chemotherapeutic process for treating tumour cells, wherein liposomes are used as drug carriers.

In the process of this invention it is possible to prepare, in simple manner and without using complicated apparatus, aqueous phases which contain small unilamellar liposomes (SUL) with a diameter of about 200 to 600 Å, and large unilamellar liposomes (LUL) with a diameter of about 600 to 3000 Å. Small unilamellar liposomes can be separated from large unilamellar liposomes by means of suitable separating methods, e.g. by gel filtration or in an ultrafiltration cell.

Claim

1. A process for the preparation of unilamellar liposomes in aqueous phase, which comprises dispersing a homogeneous mixture of an anionic surfactant and a lipid, in aqueous phase, at a concentration lower than the critical micelle concentration (cmc) of the surfactant in the

.../2

particular phase and, if necessary, neutralising the aqueous phase so obtained and, if desired, enriching and/or separating the resultant unilamellar liposomes.

2. A process according to claim 1, which comprises dispersing a homogeneous mixture of an anionic or cationic surfactant.

17402/83

COMMONWEALTH OF AUSTRALIA

Patents Act 1952-1969

CONVENTION APPLICATION FOR A PATENT

(1) Here insert (in full) Name or Names of Applicant or Applicants, followed by Address (es).

I/We (1) CIBA-GEIGY AG,

of Klybeckstrasse 141, 4002 Basle, Switzerland

(2) Here insert Title of Invention.

hereby apply for the grant of a Patent for an invention entitled: (2)

LIPIDS AND TENSIDS IN AQUEOUS PHASE

(3) Here insert number(s) of basic application(s)

which is described in the accompanying complete specification. This application is a Convention application and is based on the application numbered (3)

4597/82-1

(4) Here insert Name of basic Country or Countries, and basic date or dates.

for a patent or similar protection made in (4) Switzerland

on 29th July 1982

~~My~~ Our address for service is Messrs. Edwd. Waters & Sons, Patent Attorneys. 50 Queen Street, Melbourne, Victoria, Australia.

28 JUL 1983

DATED this 27th day of July 1983.

(5) Signature(s) of Applicant(s) or Seal of Company and Signatures of its Officers as prescribed by its Articles of Association

CIBA-GEIGY AG

by

W. F. Dancer

Reg'd. Patent Attorney

To:

AU Case 4-14035/+ FORM 8

COMMONWEALTH OF AUSTRALIA

17402/83 Patents Act 1952 - 1969

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION FOR A PATENT

In support of the Convention Application made by CIBA-GEIGY AG for a patent for an invention entitled:

Lipids and tensids in aqueous phase

We, Arnold Seiler and) of CIBA-GEIGY AG, Klybeckstrasse 141,
Ernst Altherr) 4002 Basle, Switzerland
do solemnly and sincerely declare as follows:

1. We are authorised by the applicant for the patent to make this declaration on its behalf.

2. The basic application(s) as defined by Section 141 on the Act was made in Switzerland on July 29, 1982

by CIBA-GEIGY AG, 4002 Basle, Switzerland.

3. Helmut Hauser, Schwarzbachstrasse 91, 8713 Uerikon, Switzerland

is the actual inventor of the invention and the facts upon which the applicant is entitled to make the application are as follows: The said applicant is the assignee of the actual inventor.

4. The basic application referred to in paragraph 2 of this Declaration was the first application made in a Convention country in respect of the invention the subject of the application.

DECLARED at Basle, Switzerland on July 11, 1983

CIBA-GEIGY AG

pp

To: The Commissioner of Patents.

Form 10

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952-69

COMPLETE SPECIFICATION

(ORIGINAL)

Class Int. Class

Application Number: 17402/83
Lodged:

Complete Specification Lodged:
Accepted:
Published:

Priority :

Related Art :

Name of Applicant : CIBA-GEIGY AG

Address of Applicant : Klybeckstrasse 141, 4002 Basle, Switzerland

Actual Inventor: HELMUT HAUSER

Address for Service : EDWD. WATERS & SONS,
50 QUEEN STREET, MELBOURNE, AUSTRALIA, 3000.

Complete Specification for the invention entitled:

LIPIDS AND TENSIDS IN AQUEOUS PHASE

The following statement is a full description of this invention, including the best method of performing it known to :- us

Case 4-14035/+

Lipids and tensids in aqueous phase

The present invention relates to a process for the preparation of unilamellar liposomes in aqueous phase.

Liposomes have been described in the literature in a wide range of publications, and many investigations are concerned with their structure and use. A distinction is made between unilamellar liposomes having a double layer of lipids and multilamellar liposomes having a number of double layers of lipids of onion-like structure.

Unilamellar liposomers have a spherical shell and a diameter of about 200 to 50,000 Å, preferably of about 200 to 30,000 Å. The spherical shell consists of a double layer of the lipid components, e.g. amphiphatic lipids such as phospholipids, e.g. phosphatidic acid, lecithin or cephalin, with or without neutral lipids, e.g. cholesterol. This double layer surrounds a cavity which contains an aqueous phase.

There is great interest in the therapeutic use of liposomes as carriers for a very wide range of active ingredients. Accordingly, liposomes have been proposed as carriers for proteins, e.g. antibodies or enzymes, hormones, vitamins or genes or, for analytical purposes, as carriers for marker compounds. For example, US patent 3 933 754 describes a chemotherapeutic process for treating tumour cells, wherein liposomes are used as drug carriers.

The drug is encapsulated either during the formation of the liposomes or subsequently by diffusion. The preparation of liposomes and the encapsulation of the drug can be effected by different methods, a survey of which may be found in the article "Liposomes - Problems

and promise as selective drug carriers" by Stanley B. Kaye, Cancer Treatment Reviews (1981), 8, pp. 27-50. Further methods of preparing liposomes for encapsulating drugs are also described by Barenholz et al. in Biochemistry, Vol. 16, No. 12, 2806-2810, and also in German Offenlegungsschrift specifications 28 19 855, 29 02 672, 25 32 317 and 28 42 608, in US patent 4 053 585, and in European patent application 36 676.

In the prior art methods, the lipid components, e.g. phospholipids such as phosphatidic acid, lecithin or cephalin, with or without neutral lipids, e.g. cholesterol, are dissolved in an organic solvent, e.g. chloroform or benzene. After stripping off the solvent, there remains a homogeneous layer, e.g. a film, of the particular lipid components. The lipid components are subsequently dispersed in an aqueous phase which contains the appropriate drug, e.g. by shaking. Unilamellar liposomes which encapsulate the drug are formed in the course of the subsequent treatment with ultrasonic irradiation.

In the process of this invention it is possible to prepare, in simple manner and without using complicated apparatus, aqueous phases which contain small unilamellar liposomes (SUL) with a diameter of about 200 to 600 Å, and large unilamellar liposomes (LUL) with a diameter of about 600 to 3000 Å. Small unilamellar liposomes can be separated from large unilamellar liposomes by means of suitable separating methods, e.g. by gel filtration or in an ultrafiltration cell.

The present invention relates to a process for the preparation of unilamellar liposomes, which comprises dispersing a homogeneous mixture of an ionic surfactant and a lipid, in aqueous phase, at a concentration lower than the critical micelle concentration (cmc) of the surfactant in the particular phase and, if necessary, neutralising the aqueous phase so obtained and, if desired, enriching and/or separating the resultant unilamellar liposomes.

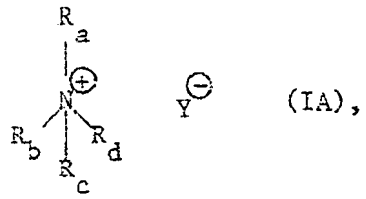
Throughout this specification, the general terms employed preferably have the meanings set forth below.

The term "lower" used to qualify organic radicals, e.g. lower alkyl, lower alkylene, lower alkoxy, lower alkanoyl etc., denotes that such radicals, unless otherwise expressly defined, contain from 1 to 7, preferably 1 to 4, carbon atoms.

The preparation of the homogeneous mixture of an ionic surfactant and a lipid is effected in a manner which is known per se and is described in the section entitled "Preparation of the homogeneous layer of the lipid components."

An ionic surfactant will be understood as meaning a cationic or anionic surfactant.

A cationic surfactant is e.g. a compound of the formula



(IA),

wherein R_a is an unsubstituted or substituted hydrocarbon radical, R_b is lower alkyl, phenyl-lower alkyl or hydroxy, R_c and R_d are lower alkyl, or R_b and R_c, together with the nitrogen atom to which they are attached, form an aliphatic heterocyclic ring system which may be substituted at a carbon atom, and R_d is lower alkyl, or R_b, R_c and R_d, together with the nitrogen atom to which they are attached, form an aromatic heterocyclic ring system, and $Y^{\ominus}$ is an anion.

In a cationic surfactant of the formula (IA), an unsubstituted or substituted aliphatic hydrocarbon radical R_a is for example lower alkyl substituted by aryloxy-lower alkoxy, or is straight chain or

branched alkyl containing 7 to 22, preferably 12 to 20, carbon atoms, or alkenyl containing 8 to 20, preferably 12 to 20, carbon atoms and 1 to 4 double bonds.

Aryl in arlyoxy-lower alkoxy is for example phenyl which may be mono- or disubstituted by straight chain C_1-C_4alkyl, e.g. methyl, ethyl or n-propyl, or by branched C_3-C_{10}alkyl, e.g. isobutyl, tert-butyl, amyl, neopentyl, 2- or 3-methylpentyl, 2,2- or 2,3-dimethylbutyl, 2- or 3-methylhexyl, 3-ethylpentyl, 2,2-, 2,3-, 2,4- or 3,3-dimethylpentyl, 4-methylheptyl, 2,2,2-, 2,2,4-, 2,3,3- or 2,3,4-trimethylpentyl, 1,1,3,3-tetramethylbutyl or 2,2,3,3-tetramethylbutyl.

Lower alkoxy in aryloxy-lower alkoxy is for example methoxy, ethoxy, n-propoxy or n-butoxy.

R_a as lower alkyl substituted by aryloxy-lower alkoxy is for example aryloxy-lower alkoxymethyl or 2-aryloxy-lower alkoxyethyl, e.g. aryloxy-methoxymethyl, 2-aryloxymethoxyethyl, 2-aryloxyethoxymethyl or 2-(2-aryloxyethoxy)ethyl, e.g. phenoxymethoxymethyl, 2-phenoxymethoxyethyl, 2-phenoxyethoxymethyl, 2-(2-phenoxyethoxy)ethyl, 2-, 3- or 4-methylphenoxymethyl, 2-(2-methylphenoxymethoxy)ethyl, 2-(3-methylphenoxymethoxy)ethyl, 2-(4-methylphenoxymethoxy)ethyl, 2-(2-methylphenoxy)ethoxymethyl, 2-(3-methylphenoxy)ethoxymethyl, 2-(4-methylphenoxy)ethoxymethyl, 2-[2-(2-methylphenoxy)ethoxy]ethyl, 2-[2-(3-methylphenoxy)ethoxy]ethyl, 2-[2-(4-methylphenoxy)ethoxy]ethyl, 4-(1,1,3,3-tetramethylbutyl)phenoxymethoxymethyl, 2-[4-(1,1,3,3-tetramethylbutyl)phenoxymethoxy]ethyl, 2-[4-(1,1,3,3-tetramethylbutyl)-phenoxy]ethoxymethyl, 2-[2-(4-(1,1,3,3-tetramethylbutyl)phenoxy)-ethoxy]ethyl, 2-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxymethoxyethyl, 2-[2-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxymethoxy]ethyl, 2-[3-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxymethoxy]ethyl, 2-[2-(2-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy)]ethoxymethyl, 2-[2-(3-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxymethyl, 2-[2-(2-methyl-4-(1,1,3,3-tetramethylbutyl)-phenoxy)ethoxy]ethyl or 2-[2-(3-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy)ethoxy]ethyl.

R_a as lower alkyl substituted by aryloxy-lower alkoxy is preferably 2-[2-(2-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy)ethoxy]ethyl and 2-[2-(3-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy)ethoxy]ethyl.

R_a as straight chain or branched alkyl of 7 to 22, preferably 12 to 20, carbon atoms, is for example n-heptyl, 2-methylhexyl, 3-methylhexyl, 3-ethylpentyl, 2,2-, 2,3-, 2,4- or 3,3-dimethylpentyl, n-octyl, 4-methylheptyl, 2,2,3-, 2,2,4-, 2,3,3-, 2,3,4-trimethylpentyl, n-nonyl, n-decyl, n-undecyl, n-dodecyl (lauryl), n-tridecyl, n-tetradecyl (myristyl), n-pentadecyl, n-hexadecyl (cetyl), n-heptadecyl, n-octadeyl (stearyl), n-nonadecyl or n-eicosyl (arachinyl).

Straight chain alkyl containing an even number of 12 to 20 carbon atoms is preferred, e.g. n-dodecyl (lauryl, n-tetradecyl (myristyl), n-hexadecyl (cetyl), n-octadecyl (stearyl) or n-eicosyl (arachinyl).

R_a as alkenyl containing 8 to 20, preferably 12 to 20, carbon atoms and 1 to 4 double bonds is for example octen-1-yl, nonen-1-yl, decen-1-yl, undecen-1-yl, dodecen-1-yl, 9-cis-dodecenyl (lauroleyl), tridecen-1-yl, tetradecen-1-yl, 9-cis-tetradecenyl (myristoleyl), pentadecen-1-yl, hexadecen-1-yl, 9-cis-hexadecenyl (palmitoleinyl), heptadecen-1-yl, octadecen-1-yl, 6-cis-octadecenyl (petroselinyl), 6-trans-octadecenyl (petroselaidinyl), 9-cis-octadecenyl (oleyl), 9-trans-octadecenyl (elaidinyl), 9-cis-12-trans-octadecadienyl (linoleyl), 9-cis-11-trans-13-trans-octadecatrienyl (α-eleostearinyl), 9-cis-11-trans-13-trans-octadecatrienyl (β-eleostearinyl), 9-cis-12-cis-15-cis-octadecatrienyl (linolenyl), 9-, 11-, 13-, 15-octadecatetraenyl (parinaryl), nonadecen-1-yl, eicosen-1-yl, 9-cis-eicosenyl (gadoleinyl), 5-, 11-, 14-eicosatrienyl or 5-, 8-, 11-, 14-eicosatetraenyl (arachidonyl).

Alkenyl containing 12 to 20 carbon atoms and one double bond is preferred, e.g. 9-cis-dodecenyl (lauroleyl), 9-cis-tetradecenyl (myristoleyl), 9-cis-hexadecenyl (palmitoleinyl), 6-cis-octadecenyl (petroselinyl), 6-trans-octadecenyl (petroselaidinyl), 9-cis-octadecenyl (oleyl), 9-trans-octadecenyl (elaidinyl) or 9-cis-eicosenyl (gadoleinyl).

R_b, R_c or R_d as lower alkyl is for example methyl or ethyl. R_b as phenyl-lower alkyl is for example benzyl or 2-phenylethyl.

An aliphatic heterocyclic ring system formed by R_b and R_c together with the nitrogen atom to which they are attached is for example a monocyclic 5- or 6-membered azacyclyl, oxaazacyclyl or thiazacyclyl radical, e.g. piperidino, morpholino or thiamorpholino. Substituents of this heteroring are the substituents R_a and R_d at the nitrogen and lower alkyl, e.g. methyl, ethyl, n-propyl or n-butyl, at a carbon atom.

A heterocyclic ring system formed by R_b and R_c together with the nitrogen atom and substituted at a carbon atom by lower alkyl is e.g. 2-, 3- or 4-methylpiperidinio, 2-, 3- or 4-ethylpiperidinio or 2- or 3-methylmorpholinio.

An aromatic heterocyclic ring system formed by R_b, R_c and R_d together with the nitrogen atom is e.g. a monocyclic 5- or 6-membered azacyclyl, diazacyclyl, oxaazacyclyl or thiazacyclyl radical, e.g. pyridinio, imidazolinio, oxazolinio or thiazolinio, or for example a monoaza-bicyclyl radical which is fused to a benzene ring, e.g. quinolinio or isoquinolinio. Substituents of this hetero-ring are the radical R_a at the nitrogen and lower alkyl at a carbon atom, e.g. methyl or ethyl, hydroxy-lower alkyl, e.g. hydroxymethyl or 2-hydroxyethyl, oxo, hydroxy or halogen, e.g. chlorine or bromine.

A heterocyclic ring system formed by R_b, R_c and R_d and substituted at a carbon atom by the above mentioned radicals is for example 2- or 4-lower alkylpyridinio, e.g. 2- or 4-methylpyridinio or 2- or 4-ethylpyridinio, di-lower alkylpyridinio, e.g. 2,6-dimethylpyridinio, 2-methyl-3-ethylpyridinio, 2-methyl-4-ethylpyridinio, 2-methyl-5-ethylpyridinio or 2-methyl-6-ethylpyridinio, 2-, 3- or 4-halopyridinio, e.g. 2-, 3- or 4-chloropyridinio or 2, 3- or 4-bromopyridinio, 2-lower alkylimidazolinio, 2-lower alkyloxazolinio or 2-lower alkylthiazolinio, e.g. 2-methylimidazolinio or 2-ethylimidazolinio, 2-ethyl- or 2-methyloxazolinio, 2-methyl- or 2-ethylthiazolinio or 2-lower alkyl-8-haloquinolinio, e.g. 2-methyl-8-chloroquinolinio.

An anion $Y^{\ominus}$ is for example a halide ion, e.g. the fluoride, chloride or bromide ion, a lower alkanoate, e.g. the formate or acetate ion, the hydrogen sulfate ion, a lower alkylsulfate ion, e.g. the methyl or ethyl sulfate ion, a lower alkylsulfonate ion, e.g. the methyl sulfonate ion, or an arylsulfonate ion, e.g. the phenyl sulfonate ion or the toluene sulfonate ion. An anion $Y^{\ominus}$ is preferably a halide ion, e.g. the chloride or bromide ion.

A cationic surfactant of the formula IA is preferably N-benzyl N,N-dimethyl-N-2-[2-(4-(1,1,3,3-tetramethylbutyl)phenoxy)ethoxy]ethyl-ammonium chloride, N-benzyl N,N-dimethyl-N-2-[2-(3-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy)ethoxy]ethylammonium chloride (methyl-benzethonium chloride), n-dodecyltrimethylammonium chloride or bromide, trimethyl n-tetradecylammonium chloride or bromide, n-hexadecyl-trimethylammonium chloride or bromide (cetyltrimethylammonium chloride or bromide). trimethyl n-octadecylammonium chloride or bromide, ethyl n-dodecyldimethylammonium chloride or bromide, ethyldimethyl n-tetradecylammonium chloride or bromide, ethyl n-hexadecyldimethylammonium chloride or bromide, ethyldimethyl n-octadecylammonium chloride or bromide, n-alkyl benzyldimethylammonium chloride or bromide (benzalkonium chloride or bromide), e.g. benzyl n-dodecyldimethylammonium chloride or bromide, benzyl n-hexadecyldimethylammonium chloride or

bromide or benzyldimethyl n-octadecylammonium chloride or bromide, N-(n-decyl)pyridinium chloride or bromide, N-(n-dodecyl)pyridinium chloride or bromide, N-(n-tetradecyl)pyridinium chloride or bromide, N-(n-hexadecyl)pyridinium chloride or bromide (cetylpyridinium chloride or bromide), or N-(n-octadecyl)pyridinium chloride or bromide, or a mixture of these surfactants.

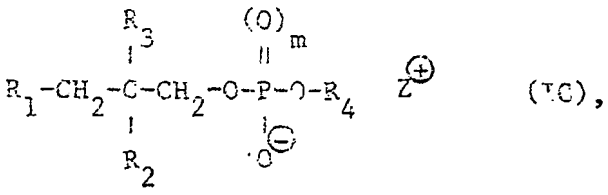
An anionic surfactant is for example

a) a compound of the formula

$$[R_a-(O-A)_m-B]^{\ominus}Z^{\oplus} \qquad (IB)$$

wherein R_a is an unsubstituted or substituted hydrocarbon radical, A is lower alkylene, m is 0 (direct bond) or 1, B is the sulfonate or sulfate group and $Z^{\oplus}$ is a monovalent cation, or

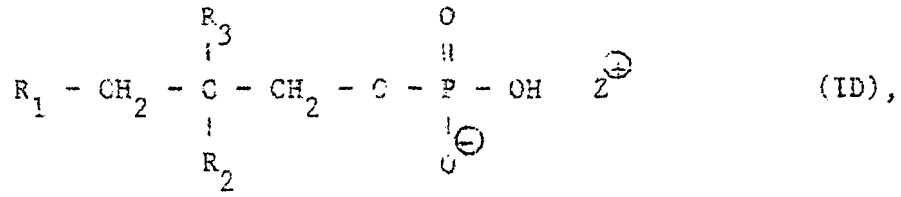
b) a compound of the formula



$$Z^{\oplus} \qquad (IC),$$

wherein m is 0 or 1, one of R_1 and R_2 is hydrogen, hydroxy or lower C_1-C_4alkyl, and the other is alkyl, alkenyl, alkoxy, alkenyloxy or acyloxy, each of 10 to 20 carbon atoms, R_3 is hydrogen or lower C_1-C_4alkyl, and R_4 is unsubstituted or substituted lower C_1-C_7alkyl, a carbohydrate radical of 5 to 12 carbon atoms or, if both R_1 and R_2 are hydrogen or hydroxy, is a steroid radical, and $Z^{\oplus}$ is a monovalent cation, or

c) a compound of the formula



(ID),

wherein R_1, R_2, R_3 and $Z^{\oplus}$ are as defined for formula (IC).

In an anionic surfactant of the formula (IB), the unsubstituted or substituted hydrocarbon radical R_a is as defined for formula (IA) and is preferably straight chain or branched alkyl containing 7 to 22, preferably 12 to 20, carbon atoms, and alkenyl containing 6 to 20, preferably 12 to 20, carbon atoms and 1 to 4 double bonds.

In an anionic surfactant of the formula (IB), R_a is preferably straight chain alkyl containing an even number of 12 to 20 carbon atoms, for example n-dodecyl (lauryl), n-tetradecyl (myristyl), n-hexadecyl (cetyl), n-octadecyl (stearyl) or n-eicosyl (arachinyl), or is alkenyl containing 12 to 20 carbon atoms and one double bond, for example 9-cis-dodecenyl (lauroleyl), 9-cis-tetradecenyl (myristoleyl), 9-cis-hexadecenyl (palmitoleinyl), 6-cis-octadecenyl (petroselinyl), 6-trans-octadecenyl (petroselaidinyl), 9-cis-octadecenyl (oleyl), 9-trans-octadecenyl (elaidinyl) or 9-cis-eicosenyl (gadoleinyl).

A as lower alkylene is for example methylene, ethylene, n-propylene or n-butylene.

The cation $Z^{\oplus}$ is an alkali metal cation, e.g. the lithium, sodium or potassium cation, or is a tetra-lower alkylammonium cation, e.g. tetramethylammonium.

An anionic surfactant of the formula IB is preferably an alkali metal alkyl sulfate (m = 0), e.g. sodium or potassium n-dodecyl (lauryl) sulfate, sodium or potassium n-tetradecyl (myristyl) sulfate, sodium

or potassium n-hexadecyl (cetyl) sulfate or sodium or potassium n-octadecyl (stearyl) sulfate, an alkali metal alkyl ether sulfate (m = 1), e.g. sodium or potassium m-dodecyloxyethyl sulfate, sodium or potassium n-tetradecyloxyethyl sulfate, sodium or potassium n-hexadecyloxyethyl sulfate or sodium or potassium n-octadecyloxyethyl sulfate, or an alkali metal alkane sulfonate, e.g. sodium or potassium n-dodecane sulfonate, sodium or potassium n-tetradecane sulfonate, sodium or potassium n-hexadecane sulfonate or sodium or potassium n-octadecane sulfonate.

In an anionic surfactant of the formula IC, R_1, R_2 or R_3 as lower C_1-C_4alkyl is preferably methyl, and also ethyl, n-propyl or n-butyl.

R_1 or R_2 as alkyl is preferably n-decyl, n-undecyl, n-dodecyl (lauryl), n-tridecyl, n-tetradecyl (myristyl), n-pentadecyl, n-hexadecyl (cetyl), n-octadecyl (stearyl) and n-eicosyl (arachinyl).

R_1 or R_2 as alkenyl is preferably 9-cis-dodecenyl (lauroleyl), 9-cis-tetradecenyl (myristoleyl), 9-cis-hexadecenyl (palmitoleinyl), 6-cis-octadecenyl (petroselinyl), 6-trans-octadecenyl (petroselaidinyl), 9-cis-octadecenyl (oleyl), 9-trans-octadecenyl (elaidinyl) or 9-cis-eicosenyl (gadoleinyl).

R_1 or R_2 as alkoxy is preferably n-decyloxy, n-dodecyloxy (lauryloxy), n-tetradecyloxy (myristyloxy), n-hexadecyloxy (cetyloxy), n-octadecyloxy (stearyloxy) or n-eicosyloxy (arachinyloxy).

R_1 or R_2 as alkenyloxy is preferably 9-cis-dodecenyloxy (lauroleyloxy), 9-cis-tetradecenyloxy (myristoleyloxy), 9-cis-hexadecenyloxy (palmitoleinyloxy), 6-cis-octadecenyloxy (petroselinyloxy), 6-trans-octadecenyloxy (petroselaidinyloxy), 9-cis-octadecenyloxy (oleyloxy), 9-trans-octadecenyloxy (elaidinyloxy) or 9-cis-eicosenyl (gadoleinyloxy).

R_1 or R_2 as acyloxy is e.g. alkanoyloxy or alkenoyloxy.

R_1 or R_2 as alkanoyloxy is preferably n-decanoyloxy, n-dodecanoyloxy (lauroyloxy), n-tetradecenoyloxy (myristoyloxy), n-hexadecanoyloxy (palmitoyloxy), n-octadecanoyloxy (stearoyloxy) or n-eicosoyloxy (arachinoyloxy).

R_1 or R_2 as alkenoyloxy is preferably 9-cis-dodecenyloxy (lauroleoyloxy), 9-cis-tetradecenoyloxy (myristoleoyloxy), 9-cis-hexadecenoyloxy (palmitoleinoyloxy), 6-cis-octadecenoyloxy (petroselinoyloxy), 6-trans-octadecenoyloxy (petroselaidinoyloxy), 9-cis-octadecenoyloxy (oleoyloxy), 9-trans-octadecenoyloxy (elaidinoyloxy) or 9-cis-eicosenoyl (gadoleinoyloxy).

R_4 as lower C_1-C_7alkyl is e.g. methyl, ethyl, isopropyl, n-propyl, isobutyl or n-butyl, and may be substituted by acidic groups, e.g. carboxyl or sulfo, by acidic and basic groups, e.g. carboxyl and amino, in which case the amino group is in the α-position relative to the carboxyl group, by free or etherified hydroxyl groups, where two etherified hydroxyl groups may be linked to each other through a divalent hydrocarbon radical, e.g. by methylene, ethylene, ethylidene, 1,2-propylene or 2,2-propylene, by halogen, e.g. chlorine or bromine, by lower alkoxycarbonyl, e.g. methoxycarbonyl or ethoxycarbonyl, or by lower alkanesulfonyl, e.g. methanesulfonyl.

R_4 as substituted C_1-C_7alkyl is preferably carboxy-lower alkyl, e.g. carboxymethyl, 2-carboxyethyl or 3-carboxy-n-propyl, ω-amino-ω-carboxy-lower alkyl, e.g. 2-amino-2-carboxyethyl or 3-amino-3-carboxy-n-propyl, hydroxy-lower alkyl, e.g. 2-hydroxyethyl or 2,3-dihydroxypropyl, lower alkoxy-lower alkyl, e.g. methoxy, methyl or ethoxymethyl, 2-methoxyethyl or 3-methoxy-n-propyl, lower alkylenedioxy-lower alkyl, e.g. 2,3-ethylenedioxypropyl or 2,3-(2,2-propylene)dioxypropyl, or halo-lower alkyl, e.g. chloromethyl or bromomethyl, 2-chloroethyl or 2-bromoethyl, 2- or 3-chloro-n-propyl

or 2- or 3-bromo-n-propyl.

R_4 as a carbohydrate radical of 5 to 12 carbon atoms is e.g. a natural monosaccharide radical which is derived from a pentose or hexose in the form of an aldose or a ketose.

A pentose in the form of an aldose is e.g. D-ribose, D-arabinose, D-xylose or D-lyxose. A pentose in the form of a ketose is e.g. D-ribulose or D-xylulose. A hexose in the form of an aldose is e.g. D-allose, D-altrose, D-glucose, D-mannose, D-galactose or D-talose. A hexose in the form of a ketose is e.g. D-psicose, D-fructose, D-sorbose or D-tagatose.

A hexose is preferably in cyclic form, e.g. in the form of a pyranose (aldose), e.g. α- or β-D-glucopyranose, or a furanose, e.g. α- or β-D-fructose. The pyranosyl radical is preferably esterified with the phosphatidyl group through the hydroxy group in the 1- or 6-position, and the furanosyl radical is esterified with the phosphatidyl group through the hydroxyl group in the 1- or 5-position (m = 1).

A carbohydrate radical R_4 of 5 to 12 carbon atoms is also a natural disaccharide radical, e.g. a disaccharide radical which is formed from two hexoses by condensation of two aldoses, e.g. D-glucose or D-galactose, or of an aldose, e.g. D-glucose, with a ketose, e.g. fructose.

Disaccharides formed from two aldoses, e.g. lactose or maltose, are preferably esterified with the phosphatidyl group through the hydroxyl group which is in the 6-position of the particular pyranosyl radical. Disaccharides formed from an aldose and a ketose, e.g. saccharose, are preferably esterified with the phosphatidyl group through the hydroxyl group which is in the 6-position of the pyranosyl radical or in the 1-position of the furanosyl radical (m = 1).

A carbohydrate radical R_4 of 5 to 12 carbon atoms is further a derived mono- or disaccharide radical, wherein e.g. the aldehyde group and/or one or two terminal hydroxyl groups are oxidised to carboxyl groups, and is e.g. a D-gluconic, D-glucaric or D-glucoronic acid radical which is preferably in the form of a cyclic lactone radical. Likewise, the aldehyde or keto group of a derived mono- or disaccharide radical can be reduced to hydroxyl groups, e.g. inositol, sorbitol or D-mannitol, or hydroxyl groups can be replaced by hydrogen, e.g. desoxy sugar, e.g. 2-desoxy-D-ribose, L-rhamnose or L-fucose, or by amino groups, e.g. amino sugar, e.g. D-glucosamine or D-galactosamine.

A carbohydrate radical R_4 can also be a fission product formed by reacting one of the mono- or disaccharides mentioned above with a strong oxidising agent, e.g. periodic acid.

A steroid radical R_4 is e.g. a sterol radical which is esterified with the phosphatidyl group through the hydroxyl group which is in the 3-position of the steroid skeleton (m = 1).

A sterol radical is e.g. lanosterol, sitosterol, coprostanol, cholestanol, glycocholic acid, ergosterol or stigmasterol, but is preferably cholesterol.

If R_4 is a steroid radical, R_1 and R_2 are preferably hydroxyl and R_3 is hydrogen.

$Z^{\oplus}$ is as defined for formula IB and is preferably sodium or potassium.

In an anionic surfactant of the formula IC, m is preferably 1, R_1 is alkyl, e.g. n-dodecyl, (lauryl), n-tridecyl, n-tetradecyl (myristyl), n-pentadecyl, n-hexadecyl (cetyl), n-heptadecyl or n-octadecyl (stearyl), alkoxy, e.g. n-dodecyloxy (lauryloxy),

n-tetradecyloxy (myristyloxy), n-hexadecyloxy (cetyloxy), or n-octadecyloxy (stearyloxy), acyloxy, e.g. lauroyloxy, myristoyloxy, palmitoyloxy or stearyloxy, R_2 is hydrogen or hydroxy, R_3 is hydrogen or lower alkyl, e.g. methyl, R_4 is lower alkyl, e.g. methyl or ethyl, lower alkyl substituted by acid and basic groups, e.g. carboxy and amino, e.g. ω-amino-ω-carboxy-lower alkyl, e.g. 2-amino-2-carboxyethyl or 3-amino-3-carboxy-n-propyl, hydroxy-lower alkyl, e.g. 2-hydroxyethyl or 2,3-hydroxypropyl, lower alkylenedioxy-lower alkyl, e.g. 2,3-ethylenedioxypropyl or 2,3-(2,2-propylene)dioxypropyl, halo-lower alkyl, e.g. 2-chloroethyl or 2-bromoethyl, a carbohydrate radical of 5 to 12 carbon atoms, e.g. inositol, or a steroid radical, e.g. a sterol such as cholesterol, and $Z^{\oplus}$ is sodium or potassium.

An anionic surfactant of the formula IC is preferably the sodium or potassium salt of lysophosphatidylserine, e.g. the sodium or potassium salt of beef brain lysophosphatidylserine or the sodium or potassium salt of a synthetic lysophosphatidylserine, e.g. sodium or potassium 1-myristoyllysophosphatidylserine or sodium or potassium 1-palmitoyllysophosphatidylserine, or the sodium or potassium salt of lysophosphatidyl glyerol.

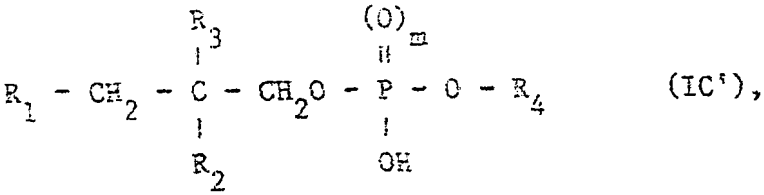
In an anionic surfactant of the formula ID, R_1, R_2, R_3 and $Z^{\oplus}$ are as defined for formula IC. The cation $Z^{\oplus}$ is preferably sodium or potassium. The hydrogen atom at the phosphate group may be replaced by a second cation $Z^{\oplus}$ or by the magnesium ion.

In an anionic surfactant of the formula ID, R_1 is preferably alkyl, e.g. n-dodecyl (lauryl), n-tridecyl, n-tetradecyl (myristyl), n-pentadecyl, n-hexadecyl (cetyl, n-heptadecyl or n-octadecyl (stearyl), or alkoxy, e.g. n-dodecyloxy (lauryloxy), n-tetradecyloxy (myristyloxy), n-hexadecyloxy (cetyloxy), or n-octadecyloxy (stearyloxy), or acyloxy, e.g. lauroyloxy, myristoyloxy, palmitoyloxy

or stearoyloxy, R_2 is hydrogen or hydroxy and R_3 is hydrogen or lower alkyl, e.g. methyl, and $Z^{\oplus}$ is sodium or potassium.

An anionic surfactant of the formula ID is in particular the sodium or potassium salt of a natural phosphatidic acid, e.g. egg phosphatidic acid, the sodium or potassium salt of a natural lysophosphatidic acid, e.g. egg lysophosphatidic acid, the sodium or potassium salt of a synthetic lysophosphatidic acid. e.g. 1-lauroyl-lysophosphatidic acid, 1-myristoyllysophosphatidic acid or 1-palmitoyllysophosphatidic acid.

A lipid which is dispersed in the aqueous phase is e.g. a compound of the formula



(IC'),

wherein m, R_1, R_2, R_3 and R_4 are as defined for formula IC, and R_4 is also lower alkyl substituted by tri-lower alkylammonio, e.g. trimethylammonio, or by amino, e.g. 2-trimethylammonioethyl (cholinyl).

A suitable lipid is preferably a lipid of the formula IC', wherein m is 1, R_1 and R_2 are acyloxy, R_3 is hydrogen and R_4 is 2-trimethylammonioethyl or 2-aminoethyl. Such a lipid is e.g. a natural lecithin, e.g. egg lecithin or lecithin obtained from soybeans (R_4 is 2-trimethylammonioethyl), and a natural cephalin, e.g. egg cephalin or cephalin obtained from soybeans (R_4 is 2-aminoethyl).

Further preferred lipids are synthetic lecithins (R_4 = 2-trimethylammonioethyl) and synthetic cephalins (R_4 = 2-aminoethyl) of the formula IC', wherein R_1 and R_2 are identical acyloxy radicals such

as lauroyloxy, oleoyloxy, linoyloxy, linoleoyloxy or arachinoyloxy, e.g. dilauroyl lecithin or cephalin, dimyristoyl lecithin or cephalin, dipalmitoyl lecithin or cephalin, distearoyl lecithin or cephalin, diarachinoyl lecithin or cephalin, dioleoyl lecithin or cephalin, dilinoyl lecithin or cephalin, dilinoleoyl lecithin or cephalin, or diarachinoyl lecithin or cephalin, R_1 and R_2 are different acyloxy radicals, e.g. R_1 is palmitoyloxy and R_2 is oleoyloxy, e.g. 1-palmitoyl-2-oleoyl lecithin or cephalin, R_1 and R_2 are identical alkoxy radicals, e.g. tetradecyloxy or hexadecyloxy, e.g. ditetradecyl lecithin or cephalin, or dihexadecyl lecithin or cephalin, R_1 is alkenyl and R_2 is acyloxy, e.g. a plasmalogen (R_4 = trimethylammonioethyl), or R_1 is acyloxy, e.g. myristoyloxy or palmitoyloxy, and R_2 is hydroxy, e.g. a natural or synthetic lysolecithin or lysocephalin, e.g. 1-myristoyl lysolecithin or lysocephalin or 1-palmitoyl lysolecithin or lysocephalin, and R_3 is hydrogen.

A suitable lipid is also a lipid of the formula IC', wherein m is 1, R_1 is alkenyl, R_2 is acylamido, R_3 is hydrogen, and R_4 is a 2-trimethylammonioethyl radical (choline radical). Such a lipid is known as sphingomyelin.

A suitable lipid is furthermore a lysolecithin analogue, e.g. 1-lauroyl-1,3-propanediol-3-phosphorylcholine, a monoglyceride, e.g. monoolein or monomyristin, a cerebroside, a ganglioside or a glyceride which contains no free or etherified phosphoryl or phosphonyl groups in the 3-position. Such a glyceride is e.g. a diacylglyceride or 1-alkenyl-1-hydroxy-2-acylglyceride containing the indicated acyl and alkenyl groups, wherein the 3-hydroxy group is etherified by one of the indicated carbohydrate radicals, e.g. a galactosyl radical, e.g. a monogalactosyl glycerol.

Yet another suitable lipid is a neutral lipid which is contained in cell membranes and is soluble only in a polar organic solvent, e.g. in chloroform. Examples of neutral lipids are steroids such as oestradiol or sterol, e.g. cholesterol, β-sitosterol, desmosterol, 7-keto-cholesterol or β-cholestanol, fat-soluble vitamins such as vitamin A, e.g. vitamin A_1 or A_2, vitamin E, vitamin K such as vitamin K_1 or K_2, or vitamin D_2 or D_3.

The homogeneous mixture consists preferably of a surfactant of the formula IA, in particular N-benzyl-N,N-dimethyl-N-2-[2-(4-(1,1,3,3-tetramethylbutyl)phenoxy)ethoxy]ethylammonium chloride, N-benzyl N,N-dimethyl-N-2-[2-(3-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy)-ethoxy]ethylammonium chloride (methylbenzethonium chloride), n-dodecyl-trimethylammonium chloride or bromide, trimethyl n-tetradecyl-ammonium chloride or bromide, n-hexadecyltrimethylammonium chloride or bromide (cetyltrimethylammonium chloride or bromide), trimethyl-n-octadecylammonium chloride or bromide, ethyl n-dodecyldimethyl-ammonium chloride or bromide, ethyldimethyl-n-tetradecylammonium chloride or bromide, ethyl n-hexadecyldimethylammonium chloride or bromide, ethyldimethyl n-octadecylammonium chloride or bromide, n-alkyl benzyldimethylammonium chloride or bromide (benzalkonium chloride or bromide), e.g. benzyl n-dodecyldimethylammonium chloride or bromide, benzyldimethyl n-tetradecylammonium chloride or bromide, benzyl n-hexadecyldimethylammonium chloride or bromide or benzyl-dimethyl-n-octadecylammonium chloride or bromide, N-(n-decyl)-pyridinium chloride or bromide, N-(n-dodecyl)pyridinium chloride or bromide, N-(n-tetradecyl)pyridinium chloride or bromide, N-(n-hexa-decyl)pyridinium chloride or bromide (cetylpyridinium chloride or bromide), or N-(n-octadecyl)pyridinium chloride or bromide, or an anionic surfactant of the formula IB, in particular sodium or potassium n-dodecyl (lauryl) sulfate, sodium or potassium n-tetradecyl (myristyl) sodium or potassium n-hexadecyl (cetyl) sulfate or sodium or potassium n-octadecyl (stearyl) sulfate, sodium or potassium n-dodecyloxyethyl sulfate, sodium or potassium n-tetradecyloxyethyl

sulfate, sodium or potassium n-hexadexyloxyethyl sulfate or sodium or potassium n-octadecyloxyethyl sulfate, or an anionic surfactant of the formula IC, in particular sodium or potassium 2,2-dimethyl-3-palmitoyloxypropyl hydrogen phosphate, sodium or potassium 1-palmitoyllysophosphatidyl glycerol, sodium or potassium 1-palmitoyllysophosphatidylserine, and a lipid of the formula IC', wherein R_1 and R_2 are acyloxy, e.g. lauroyloxy, myristoyloxy, palmitoyloxy or stearoyloxy, R_3 is hydrogen and R_4 is 2-trimethylammonioethyl, e.g. a natural cephalin such as egg cephalin or cephalin or cephalin obtained from soybeans, or 2-aminoethyl, e.g. a natural lecithin such as egg lecithin or lecithin obtained from soybeans.

The surfactants and lipids containing a chiral carbon atom mentioned above and hereinafter may also be in the form of racemic mixtures or of optionally pure enantiomers.

In the homogeneous mixture, the approximate molar ratio of anionic surfactant to lipid is 0.1 to 2:1, preferably 0.8 to 1.2:1.

The homogeneous mixture, e.g. the prepared film or foam, is subsequently dispersed in an aqueous phase containing the substances to be encapsulated, e.g. agrochemicals such as pesticides, perfumes, hardeners, dyes, or pharmaceutical drugs such as peptides, e.g. muramyl peptides, in dissolved, colloidal or suspended form, and surfactants.

Dispersion is effected e.g. by shaking or stirring the aqueous phase which contains the previously prepared homogeneous mixture. The formation of unilamellar liposomes (SUL) and (LUL) takes place spontaneously (spontaneous vesiculation), i.e. without the additional supply of external energy and at a high rate. The concentration of surfactant, lipid and encapsulated compound can be increased until the critical micelle concentration (cmc) of the particular ionic

surfactant in the particular aqueous phase is attained.

Micelles are preferably formed above the critical micelle concentration. This occurrence is often detectable by the disappearance of opalescence, e.g. clarification of the aqueous phase. The cmc is a variable indicating the amount of an anionic surfactant which can be dispersed in a specific volume of water while avoiding micelle formation. The structure of the hydrophobic radical of the surfactant influences the cmc value: the longer the chain length, the lower the cmc value. Voluminous substituents in the hydrophobic radical, e.g. an aromatic radical, also lower the cmc. Functional groups, e.g. double bonds which weaken the hydrophobic character of the hydrophobic radical, increase the cmc. The cmc is further influenced by all dispersed and dissolved components present in the aqueous phase, e.g. by counterions, additional lipids, the character of the active ingredient to be encapsulated etc. The cmc value can only be determined experimentally for the particular system, namely indirectly by electrochemical methods, e.g. by conductivity measurements or potentiometric determination of the counterions using a suitable electrode, by measuring the transport number and the surface tension, by measuring colligative properties such as lowering of vapour pressure, lowering of the freezing point and osmotic pressure, measuring the density, the refractive index, the absorption of UV and IR light, solubilisation of soluble and insoluble dyes, light scattering, fluorescence polarisation and viscosity. These properties undergo a substantial change when the cmc is attained. For example, the surface tension decreases sharply as a function of the concentration of the ionic surfactant until the cmc is attained, but remains virtually constant above the cmc. Reference is made in this connection to the particulars given in H. Stache, Tensidtaschenbuch, Hanser 1981, especially on page 26, 3.1 "Methoden zur cmc-Bestimmung" and page 28, 3.2 "Abhängigkeit der cmc von verschiedenen Parametern." Specific cmc values, e.g. for dodecylpyridinium bromide, are given by J.B. Adderson and H. Taylor,

J. Colloid. Sci. 19, 495 (1964). If the cmc value is exceeded, it is possible to lower the concentration by diluting the aqueous phase with water. Reversibly unilamellar liposomes are then formed from the micelles.

Aqueous phases with a pH higher than about 8 are neutralised following dispersion, e.g. to physiological pH 7.2. Neutralisation is necessary to prevent decomposition of the active ingredient and/or the liposomes under basic conditions and to ensure the physiological tolerance of the applicable aqueous phase with the mixture of liposomes. Neutralisation is effected with a physiologically acceptable acid or a buffer solution with a pH of 7 to 8. Physiologically acceptable aicds are e.g. dilute mineral acids such as dilute hydrochloric acid, sulfuric acid or phosphoric acid, or organic acids such as lower alkanecarboxylic acids, e.g. acetic acid.

Aqueous phases containing cationic surfactants of the formula IA may show acid reaction. These phases are neutralised by adding dilute aqueous bases, e.g. dilute aqueous NaOH or KOH or a buffer solution with a pH of 7 to 8.

The process is conveniently carried out at room temperature or also at elevated temperature, e.g. up to about 60°C, and with stirring or shaking. If the limited stability of the active ingredient to be encapsulated requires it, the process is carried out with cooling and, if appropriate, in an inert gas atmosphere, e.g. in a nitrogen atmosphere. The liposomes so obtained are fairly stable in aqueous phase (up to several days). Aqueous phases containing unilamellar liposomes obtainable by the process of this invention can be made storage stable by the process described in European patent application 00 65 292.

The size of the unilamellar liposomes depends inter alia on the structure of the surfactants and of the lipid components, on the ratio of the components, on the concentration of these components in the aqueous phase, and on the amount and structure of the drug to be encapsulated. Accordingly, for example, aqueous phases containing a high concentration of small or large unilamellar liposomes can be prepared by varying the concentration of the surfactant components. In addition to SUL, large unilamellar liposomes (LUL, diameter up to 50,000 Å) are also formed. These encapsulate larger volumes per mole of lipid components employed and are suitable for encapsulating voluminous substances, e.g. viruses, bacteria or cell organellae.

The separation of SUL from LUL is accomplished by conventional separation methods such as gel filtration, e.g. with Sepharose 4B or Sephacryl as carrier, or by sedimentation of the LUL in an ultracentrifuge at 160,000 x g. For example, the LUL deposit after centrifugation for several hours, e.g. about 3 hours, in this gravitional field, whereas the SUL remain in dispersion and can be decanted. Complete separation of the LUL from the SUL is achieved after repreated centrifugation.

All liposomes having a diameter greater than 600 Å present in the aqueous phase, e.g. LUL or multilamellar liposomes, as well as non-encapsulated drugs and excess dispersed lipids, can also be separated by gel filtration, so making it possible to obtain an aqueous phase containing a fraction of SUL of relatively uniform size.

After the separation of large unilamellar liposomes (LUL) and multilamellar liposomes by one of the above methods, the formation of small unilamellar liposomes and their concentration in aqueous phase can be detected by different physical methods, e.g. by applying freeze fracture samples and thin layer samples to the electron

microscope or by X-ray diffraction, by dynamic light scattering, by mass analysis of the filtrate in an analytical ultracentrifuge, in particular by spectroscopy, e.g. in the nuclear resonance spectrum (NMR) (^{1}H, ^{13}C and ^{31}P). For example, sharp signals of narrow line within the nuclear resonance spectrum indicate the formation of unilamellar liposomes with a diameter smaller than about 1000 Å. Sharp signals at δ c. 0.89 ppm ($-CH_3$), δ c. 1.28 ppm ($-CH_2-$) and δ c 3.23 ppm ($-N(CH_3)_3$) are characteristic e.g. of unilamellar liposomes obtained by the process of this invention with phosphatidyl choline as constituent. In the nuclear resonance spectrum, such signals are typical of unilamellar liposomes and differ from mixed micelles, e.g. from phospholipids such as lecithin, and surfactants such as cetyltrimethylammonium bromide. A methyl signal at δ c. 0.89 ppm is characteristic of mixed micelles with these components, which signal is resolved to a triplet and has a substantially narrower line width than the methyl signal (singlet; also δ c. 0.89 ppm) which originates from unilamellar liposomes.

The liposomes obtainable by the process of this invention (SUL and LUL) are suitable carrier systems which, in aqueous phase, may be used for solubilising lipophilic substances, e.g. fat-soluble dyes, for stabilising substances which are sensitive to hydrolysis, e.g. prostaglandins, for encapsulating pesticides, e.g. for modifying the activity spectrum of dichlorphos, for encapsulating food additives, e.g. to modify the adsorption properties of vitamins or dyes, or for introducing encapsulated drugs, enzymes, antibodies, hormones, genes, viruses, vitamins or cell organellae into the cells of a cell culture.

Aqueous phases which contain the liposomes obtainable by the process of the invention with encapsulated durgs are delivery systems which are suitable, optionally after concentration or isolation of the liposomes, e.g. by ultracentrifugation, for therapeutic purposes for oral (p.o.), parenteral (i.v. or i.p.) or topical administration.

In oral administration, liposome-based delivery systems can protect a drug, e.g. insulin, which is unstable in the digestive tract, or improve its resorption. For oral administration, the liposome-containing aqueous phase can be mixed with pharmaceutically acceptable diluents or carriers or with conventional additives such as dyes or flavourings, and administered as a syrup or in the form of capsules.

For parenteral administration, liposome-based delivery systems can prolong the retention time e.g. of desferrioxamin (q.v. R.A. Guilemette et al., Lif Sci. 22 (4), 313-320, 1978) or gentamycin (q.v. W.M. Scheld et al., Clin. Res. 26, No. 1, 59 A, 1978), in an organism. The retention time of entrapped chelating agents, e.g. EDTA (ethylenediamintetraacetic acid), in organisms is prolonged in the same manner, so that heavy metals can be removed by chelation especially from the liver, spleen or kidneys (q.v. Rahmann et al., Science, Vol. 180, 300-302, 1973, and J. Lab. Clin. Med. 640-647, 1974). With liposome-based delivery systems it is possible to enrich drugs in the myocardium (q.v. Landesmann et al., Science, Vol. 198, 737-738, 1977). It is possible to enrich antiflammatory drugs, e.g. cortisol (q.v. Nature 271, No. 5643, 372-73, 1978) or protease inhibitors (q.v. Anal. Biochem. 89, No. 2, 400-07, 1978) in the synovial fluid, and cytostatic drugs in tumour tissue (q.v. the article entitled "Liposomes - Problems and promise as selective drug carriers" by Stanley B. Kaye in Cancer Treatment Reviews 8, 27-50, 1981, and the many references cited therein). Many chemotherapeutic drugs employed in cancer therapy are less toxic and better tolerated if they are encapsulated in liposomes, e.g. liposome-encapsulated Actinomycin D (q.v. Rahmann et al., Proceedings of the Society for Experimental Biology and Medicine 146, 1173-1176, 1974), Methotrexate (q.v. L.D. Lasermann et al., Proc. Natl. Acad. Sci. 77, No. 7, 4089-93, 1980), Vinblastin, Daunomycin or cytosin-arabinoside (q.v. Mühlensiepen et al., Cancer Res. 41, No. 5, 1602-07, 1981). Liposomes can be used for introducing e.g. enzymes, peptide hormones, genes or viruses into the cytoplasma of cells in

living organisms, e.g. for introducing asparaginase (q.v. the article entitled "The Introduction of enzymes into cells by means of liposomes" by M. Finkelstein and G. Weissmann in J. Lipid Research, Vol. 19, 1978, 289-303), of amyloglucosidase (q.v. G. Gregoriadis and B.E. Ryman, Eur. J. Biochem. 24 (1972), 485-491, or neuromidase (q.v. Gregoriadis et al., Biochem. J. (1974) 140, 232-330), for bonding specific detection molecules, e.g. monoclonal antibodies, for specific introduction into defined target cells (q.v. Lesermann et al., Nature 292 (5829), 226-228, 1981), for immunostimulation as adjuvant for inoculations, e.g. against leishmaniasis (q.v. New, R.R.C. et al., Nature 272 (5648) 55-56, 1978), or for the induced release of drugs by signals such as temperature increases, e.g. in inflamed tissue, or changes in pH values. For parenteral administration, the concentrated or isolated liposomes can be suspended in a suitable carrier liquid, for example in sterile distilled water or in physiological sodium chloride solution.

Preparation of the homogeneous layer of lipid components

The homogeneous layer of lipid components can be prepared in a manner which is known per se. For example, the surfactant of the formula IA, e.g. cetylpyridinium chloride, and the lipid, e.g. egg lecithin, optionally in admixture with a lipophilic active ingredient, e.g. a protein which is encapsulated during the formation of the liposome in the lipid layer, is dissolved in an organic solvent. A homogeneous layer of lipid components consisting of a film is obtained by removing the organic solvent, most conveniently in vacuo or by blowing off with an inert gas, e.g. nitrogen.

The choice of solvent depends on the solubility of the particular lipid components therein. Examples of suitable solvents are: halogenated, aliphatic, cycloaliphatic, aromatic or aromatic-aliphatic hydrocarbons, e.g. benzene, toluene, methylene chloride or chloroform; alcohols, e.g. methanol or ethanol; lower alkanecarboxylates, e.g. ethyl acetate; ethers, e.g. diethyl ether, dioxan

or tetrahydrofuran; or mixtures of these solvents.

A homogeneous mixture can be prepared by the manner described in German Auslegeschrift 28 18 655 by lyophilisation from organic solution. The homogeneous layer is obtained as a foam.

The ionic surfactants mentioned in the description, e.g. the cationic surfactants of the formula IA and the anionic surfactants of the formula IB are known. The preparation of these surfactants is described in the standard work "Cationic Surfactants" by Eric Jungermann, Dekker, New York 1970. The annually published handbook "McCutcheon's Emulsifiers & Detergents", Manufacturing Confectioner Publishing Co., provides a survey of all commercially available anionic and cationic surfactants together with the trade names under which these surfactants are marketed by the manufacturers. The surfactants of the formulae IB and IC are known or, if novel, can be prepared in a manner known per se in accordance with the particulars given in Chapter 3 of the standard work by C.G. Knight, Liposomes, Elsevier 1981. The lipids referred to hereinbefore are known and most are commercially available.

The following Examples illustrate the invention, without implying any restriction to what is disclosed therein. Chemical displacements (δ) in the NMR spectrum are indicated in ppm.

Example 1: 10 mg of egg lecithin and 0.05 g of cetyltrimethylammonium bromide are dissolved in 2 ml of a 2:1 mixture of chloroform/methanol and this solution is concentrated in vacuo by rotary evaporation. Unilamellar liposomes are formed by dispersing the film-like residue at room temperature in 1 ml of water by shaking for 5-10 minutes. A slightly opalescent aqueous phase is obtained.

The formation of small unilamellar liposomes can be detected in the NMR spectrum by the signals δ = 1.28 (methylene), δ = 0186 (methyl) and δ = 3.25 ($-N(CH_3)_3$).

The unilamellar liposomes so obtained can be made visible in an electron microscope. The liposome dispersion is first subjected to conventional freeze-fracture. There are obtained mainly two "populations" of liposomes, which differ in their average size:

1. small unilamellar liposomes (SUL) with a diameter of about 200-600 Å and

2. large unilamellar liposomes (LUL) with a diameter of about 1000-10,000 Å.

Example 2: Following the procedure of Example 1, 10 mg of egg lecithin and an increasing amount of cetyltrimethylammonium bromide (CTAB, see table 1) are dissolved in 2 ml of a 2:1 mixture of chloroform/methanol. The solution is concentrated and the residue is dispersed in water to give an opalescent aqueous phase which consists of small (SUL) and large (LUL) unilamellar liposomes.

Table 1:

Experiment	Concentration CTAB [g/l]	Yield of SUL [%]
1	0.1	10
2	0.2	10
3	0.5	10
4	1.0	10
5	2.0	12
6	5.0	14
7	7.0	20
8	10.0	40
9	15.0	70

Example 3: In each experiment, 10 mg of egg lecithin and an increasing amount of cetylpyridinium chloride (CPC, see Table 2) or benzalkonium chloride (BAS, see Table 3) are dissolved in 2 ml of a 3:1 mixture of chloroform/methanol. The solution is concentrated in vacuo and the residue is dispersed in 1 ml of water by shaking for 5-10 minutes to give an opalescent aqueous phase which consists of small (SUL) and large (LUL) unilamellar liposomes.

Table 2:

Experiment	Concentration CPC [g/l]	Yield of SUL [%]
1	1.0	10
2	1.5	15
3	2.0	20
4	2.5	20
5	3.0	25
6	3.5	30

Table 3:

Experiment	Concentration BAC [g/l]	Yield of SUL [%]
1	0.5	2
2	1.0	5
3	2.0	5
4	3.0	10
5	5.0	15
6	10.0	60

Example 4: In each experiment, 10 mg of egg lecithin and an increasing of Texapon® N 25 (sodium lauryl ether sulfate, see Table 4), octadecylphospho-D-mannitol (OPM, see Table 5) or sodium

dodecyl sulfate (SDS, see Table 6) are dissolved in 2 ml of a 2:1 mixture of chloroform/methanol. The solution is concentrated in vacuo and the residue is dispersed in 1 ml of water by shaking for 5-10 minutes to give an opalescent aqueous phase which consists of small (SUL) and large (LUL) unilamellar liposomes.

Table 4:

Experiment	Concentration Texapon N 25 [g/l]	Yield of SUL (%)
1	1.0	2
2	2.0	5
3	3.0	5
4	4.0	10

Table 5:

Experiment	Concentration OPM [g/l]	Yield of SUL [%]
1	1.0	10
2	2.0	15
3	3.0	20

Table 6:

Experiment	Concentration OPM [g/l]	Yield of SUL [%]
1	1.0	5
2	2.0	8
3	3.0	10
4	4.0	12
5	5.0	15
6	6.0	15
7	7.0	20
8	8.0	30
9	9.0	35

Example 5: A total amount of 10 mg containing the amount indicated in Table 3 of sodium 2,2-dimethyl-3-palmitoyloxypropyl hydrogen phosphate (Table 7), sodium 1-palmitoyllysophatidyl glycerol (Table 8) and sodium 1-palmitoyllysophosphatidylserine (Table 9) and the corresponding amount of egg lecithin (lipid) are dissolved in 1 ml of a 2:1 mixture of chloroform/methanol and the solution is concentrated by rotary evaporation. The film-like residue is then dispersed in 1 ml of distilled water and the dispersion is neutralised with 0.1N sodium hydroxide solution. An opalescent aqueous phase is obtained.

Table 7:

Experiment	Concentration Surfactant [g/l]	Yield of SUL [%]
1	0.5	7
2	1.0	13
3	1.5	19
4	2.0	23
5	2.5	26
6	3.0	30
7	4.0	37
8	5.0	60
9	6.0	83
10	7.0	90
11	8.0	95
12	9.0	100
13	9.5	100

Table 8:

Experiment	Concentration Surfactant [g/l]	Yield of SUL [%]
1	1.0	6
2	1.5	10
3	2.0	15
4	2.5	17
5	3.0	20
6	3.5	25
7	4.0	27
8	4.5	30
9	5.0	33
10	6.0	40

Table 9:

Experiment	Concentration Surfactant [g/l]	Yield of SUL [%]
1	1	5
2	2	8
3	3	13
4	4	18
5	5	20
6	6	25

Example 6: 3 mg of one of the surfactants listed in Table 10 and 7 mg of egg lecithin (lipid) are dissolved in 1 ml of a 2:1 mixture of chloroform/methanol and the solution is concentrated. The film-like residue is dispersed in 1 ml of water and the dispersion is neutralised with 0.1N NaOH. An opalescent aqueous phase is obtained.

Table 10:

Surfactant	Yield [% SUL]
2-hydroxyethyl-3-palmitoyloxypropyl phosphate	20
2,2-dimethyl-3-palmitoyloxypropyl hydrogenphosphate	50
3-cetyloxypropyl-2-hydroxyethyl phosphate	29
2-bromoethylcetylphosphate	30
n-eicosyl-2,3-(2,2-propylene)dioxypropyl phosphate	18
3-stearyloxypropylhydrogen phosphate	8
2,3-dihydroxypropylmyristyl phosphate	34
3-cetyloxypropylhydrogen phosphate	19
2,3-dihydroxypropyl-n-eicosyl phosphate	8
cetyl 2,3-dihydroxypropyl phosphate	25
methyl 3-stearoyloxypropyl phosphate	45

Example 7: 20 mg (0.026 mmole) of soybean lecithin, 1 mg (0.76 umole) of N-acetylmuramyl-L-alanyl-2-(1',2'-dipalmitoyl-sn-glycero-3'-phosphoryl)ethylamide and 5 mg of n-hexadecylpyridinium chloride are dissolved in 2 ml of a 2:1 mixture of chloroform/methanol and the solution is concentrated by rotary evaporation. The film-like residue is shaken for 5 minutes in 3 ml of distilled water to give an opalescent aqueous phase. The aqueous dispersion is then buffered with 0.2 ml of a 10-fold concentrate of a phosphate-buffered isotonic solution of sodium chloride (PBS for injection purposes) to pH 7.4.

Example 8: 30 mg (0.04 mmole) of soybean lecithin, 2 mg (0.004 mmole) of flumethason 21-pivalate and 8 mg (0.002 mmole) of n-hexadecyl-pyridinium chloride are dissolved in 2 ml of a 2:1 mixture of chloroform/methanol and the solution is concentrated by rotary evaporation. The film-like residue is shaken for 5 minutes in 3 ml of distilled water to give an opalescent aqueous phase. The aqueous dispersion is then buffered to pH 7.4 as described in Example 7.

Example 9: 30 mg (0.040 mmole) of soybean lecithin and 15 mg (0.042 mmole) of Lanette E® (sodium stearyl or palmityl sulfate) are dissolved in 8 ml of a 4:1 mixture of tert.-butanol/methanol at 70°C and the solution is concentrated in vacuo. The film-like residue is shaken for 5 minutes in 3 ml of distilled water to give an opalescent aqueous phase which is buffered to pH 7.4 as described in Example 7.

Example 10: 20 mg (0.026 mmole) of soybean lecithin, 1 mg (0.76 mmole) of N-acetylmuramyl-L-alanyl-2-(1',2'-dipalmitoyl-sn-glycero-3'-phosphoryl)ethylamide and 10 mg of (0.028 mmole) of Lanette E® are dissolved in 6 ml of 4:1 mixture of tert-butanol/methanol and the solution is concentrated by rotary evaporation. The film-like residue is shaken for 5 minutes in 2 ml of distilled water to give an opalescent aqueous phase. The aqueous dispersion is filled into a stirred ultrafiltration cell (Amicon®), which, instead of the ultrafilter, is provided with an even pore filter of polycarbonate (Nucleopore®) which has a pore diameter of 0.1 um, and has been washed free of particles. The dispersion is filtered under slight overpressure and with constant addition of Dulbecco's sterile buffer solution (pH 7.4 without Ca and Mg) so that the volume in the cell does not decrease to less than 30 ml. After the passage of 0.3 litre of filtrate, all the SUL are separated and the supernatant dispersion of LUL can be filled into ampoules and used for treatment assays.

Example 11: 30 mg (0.04 mmole) of soybean lecithin. 4 mg (0.081 mmole) of flumethason 21-pivalate and 10 mg (0.028 mmole) of Lanette E® are dissolved in 6 ml of a 4:1 mixture of tert-butanol/methanol at about 70°C and the solution is concentrated by rotary evaporation. The film-like residue is shaken in 3 ml of distilled water to give an opalescent aqueous phase.

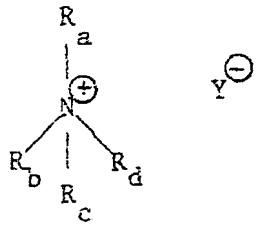
The dispersion is filled into a stirred filter cell (total volume: 100 ml) as described in Example 10 and then filtered, while adding sterile water which has been filtered until free of particles, until 500 ml of filtrate have collected. This filtrate is fed continuously into a stirred filter cell equipped with an ultrafilter, e.g. Amicon U 10 ®, and then concentrated to a volume of 30 ml. The concentrated dispersion contains small unilamellar liposomes and, after addition of Dulbecco's phosphate buffer (pH 7.4, without Ca and Mg), is filled into ampoules and used for treatment assays.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A process for the preparation of unilamellar liposomes in aqueous phase, which comprises dispersing a homogeneous mixture of an anionic surfactant and a lipid, in aqueous phase, at a concentration lower than the critical micelle concentration (cmc) of the surfactant in the particular phase and, if necessary, neutralising the aqueous phase so obtained and, if desired, enriching and/or separating the resultant unilamellar liposomes.

2. A process according to claim 1, which comprises dispersing a homogeneous mixture of an anionic or cationic surfactant.

3. A process according to claim 2, which comprises dispersing a homogeneous mixture of a cationic surfactant of the formula



(IA),

wherein R_a is an unsubstituted or substituted hydrocarbon radical, R_b is lower alkyl, phenyl-lower alkyl or hydroxy, R_c and R_d are lower alkyl, or R_b and R_c, together with the nitrogen atom to which they are attached, form an aliphatic heterocyclic ring system which may be substituted at a carbon atom, and R_d is lower alkyl, or R_b, R_c and R_d, together with the nitrogen atom to which they are attached, form an aromatic heterocyclic ring system, and $Y^{\ominus}$ is an anion, and a lipid.

4. A process according to claim 3, which comprises dispersing a homogeneous mixture of N-benzyl-N,N-dimethyl-N-2-[2-(4-(1,1,3,3-tetramethylbutyl)phenoxy)ethoxy]ethyl-

ammonium chloride, N-benzyl N,N-dimethyl-N-2-[2-(3-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy)ethoxy]ethylammonium chloride (methylbenzethonium chloride), n-dodecyltrimethylammonium chloride or bromide, trimethyl n-tetradecylammonium chloride or bromide, n-hexadecyltrimethylammonium chloride or bromide (cetyltrimethylammonium chloride or bromide), trimethyl n-octadecylammonium chloride or bromide, ethyl n-dodecyldimethylammonium chloride or bromide, ethyldimethyl n-tetradecylammonium chloride or bromide, ethyl n-hexadexyldimethylammonium chloride or bromide, ethyldimethyl n-octadecylammonium chloride or bromide, n-alkyl benzyldimethylammonium chloride or bromide (benzalkonium chloride or bromide), e.g. benzyl n-dodecyldimethylammonium chloride or bromide, benzyl n-hexadecyldimethylammonium chloride or bromide or benzyldimethyl n-octadecylammonium chloride or bromide, N-(n-decyl)pyridinium chloride or bromide, N-(n-dodecyl)pyridinium chloride or bromide, N-(n-tetradecyl)pyridinium chloride or bromide, N-(n-hexadecyl)pyridinium chloride or bromide (cetylpyridinium chloride or bromide), or N-(n-octadecyl)pyridinium chloride or bromide, or a mixture of these surfactants, and a lipid.

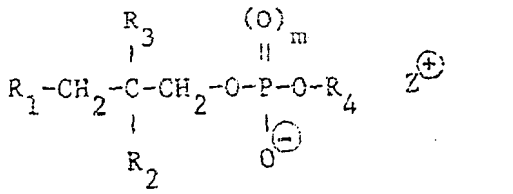
5. A process according to claim 2, which comprises dispersing a homogeneous mixture of an anionic surfactant

a) of the formula

$$[R_a-(O-A)_m-B]^{\ominus}Z^{\oplus} \qquad (IB)$$

wherein R_a is an unsubstituted or substituted hydrogen radical, A is lower alkylene, m is 0 (direct bond) or 1, B is the sulfonate or sulfate group and $Z^{\oplus}$ is a monovalent cation, or

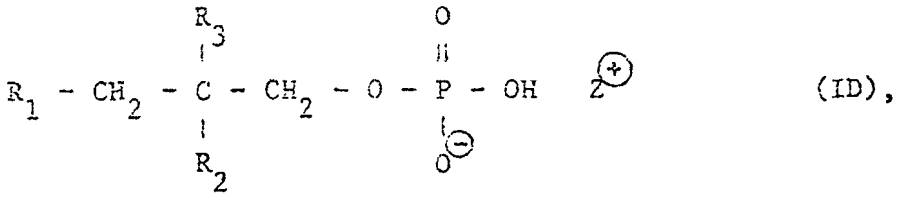
b) a compound of the formula



(IC),

wherein m is 0 or 1, one of R_1 and R_2 is hydrogen, hydroxy or lower C_1-C_4alkyl, and the other is alkyl, alkenyl, alkoxy, alkenyloxy or acyloxy, each of 10 to 20 carbon atoms, R_3 is hydrogen or lower C_1-C_4alkyl, and R_4 is unsubstituted or substituted lower C_1-C_7alkyl, a carbohydrate radical of 5 to 12 carbon atoms or, if both R_1 and R_2 are hydrogen or hydroxy, is a steroid radical, and $Z^{\oplus}$ is a monovalent cation, or

c) a compound of the formula



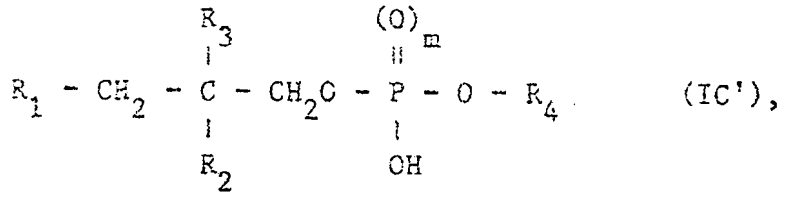
(ID),

wherein R_1, R_2, R_3 and $Z^{\oplus}$ are as defined for formula (IC), and a lipid.

6. A process according to claim 5, which comprises dispersing a homogeneous mixture containing an alkali metal alkyl sulfate (m = 0), e.g. sodium or potassium n-dodecyl (lauryl) sulfate, sodium or potassium n-tetradecyl (myristyl) sulfate, sodium or potassium n-hexadecyl (cetyl) sulfate or sodium or potassium n-octadecyl (stearyl) sulfate, an alkali metal alkyl ether sulfate (m = 1), e.g. sodium or potassium n-dodecyloxyethyl sulfate, sodium or potassium n-tetradecyloxyethyl sulfate, sodium or potassium n-hexadecyloxyethyl sulfate or sodium or potassium n-octadecyloxyethyl sulfate, or an alkali metal alkane sulfonate, e.g. sodium or potassium n-dodecane sulfonate, sodium or potassium n-tetradecane sulfonate, sodium or potassium n-hexadecane sulfonate or sodium or potassium n-octadecane sulfonate, the sodium or potassium salt of lyso-phosphatidylserine, e.g. the sodium or potassium salt of beef brain

lysophosphatidylserine or the sodium or potassium salt of a synthetic lysophosphatidylserine, e.g. sodium or potassium 1-myristoyllysophosphatidylserine or sodium or potassium 1-palmitoyl-lysophosphatidylserine, or the sodium or potassium salt of lysophosphatidylglycerol, the sodium or potassium salt of natural phosphatidic acid, e.g. egg phosphatidic acid, the sodium or potassium salt of a natural lysophosphatidic acid, e.g. egg lysophosphatidic acid, the sodium or potassium salt of a synthetic lysophosphatidic acid, e.g. 1-lauroyllysophosphatidic acid, 1-myristoyl-lysophosphatidic acid or 1-palmitoyllysophosphatidic acid, or a mixture of these surfactants and a lipid.

7. A process according to any one of claims 1 to 6, wherein the lipid is a compound of the formula



(IC'),

wherein m, R_1, R_2, R_3 and R_4 are as defined for formula IC and R_4 is also lower alkyl substituted by tri-lower alkylammonio or amino.

8. A process according to claim 7, wherein the lipid is preferably a natural lecithin, for example egg lecithin or lecithin obtained from soybeans (R_4 = 2-trimethylammonioethyl), a natural cephalin, for example egg cephalin or cephalin obtained from soybeans (R_4 = 2-aminoethyl), a synthetic lecithin (R_4 = 2-trimethylammonioethyl) or a synthetic cephalin (R_4 = 2-aminomethyl) of the formula IC', wherein R_1 and R_2 are identical acyloxy radicals such as lauroyloxy, oleoyloxy, linoyloxy, linoleoyloxy or arachinoyloxy, e.g. dilauroyl lecithin or cephalin, dimyristoyl lecithin or cephalin, dipalmitoyl lecithin or cephalin, distearoyl lecithin or cephalin, diarachinoyl lecithin or cephalin, dioleoyl lecithin or cephalin, dilinoyl lecithin or cephalin, dilinoleoyl lecithin or

cephalin, or diarachinoyl lecithin or cephalin, R_1 and R_2 are different acyloxy radicals, e.g. R_1 is palmitoyloxy and R_2 is oleoyloxy, e.g. 1-palmitoyl-2-oleoyl lecithin or cephalin, R_1 and R_2 are identical alkoxy radicals, e.g. tetradecyloxy or hexadecyloxy, e.g. ditetradecyl lecithin or cephalin, or dihexadecyl lecithin or cephalin, R_1 is alkenyl and R_2 is acyloxy, e.g. a plasmalogen (R_4 = trimethylammonioethyl), or R_1 is acyloxy, e.g. myristoyloxy or palmitoyloxy, and R_2 is hydroxy, e.g. a natural or synthetic lysolecithin or lysocephalin, e.g. 1-myristoyl lysolecithin or lysocephalin or 1-palmitoyl lysolecithin or lysocephalin, and R_3 is hydrogen.

9. A process according to any one of claims 1 to 8, which comprises dispersing a homogeneous mixture of a surfactant of the formula IA, in particular N-benzyl N,N-dimethyl-N-2-[2-(4-(1,1,3,3-tetramethylbutyl)phenoxy)ethoxy]ethylammonium chloride, N-benzyl N,N-dimethyl-N-2-[2-(3-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy)-ethoxy]ethylammonium chloride (methylbenzethonium chloride), n-dodecyl-trimethylammonium chloride or bromide, trimethyl n-tetradecyl-ammonium chloride or bromide, n-hexadecyltrimethylammonium chloride or bromide (cetyltrimethylammonium chloride or bromide), trimethyl-n-octadecylammonium chloride or bromide, ethyl n-dodecyldimethyl-ammonium chloride or bromide, ethyldimethyl-n-tetradecylammonium chloride or bromide, ethyl n-hexadecyldimethylammonium chloride or bromide, ethyldimethyl n-octadecylammonium chloride or bromide, n-alkyl benzyldimethylammonium chloride or bromide (benzalkonium chloride or bromide), e.g. benzyl n-dodecyldimethylammonium chloride or bromide, benzyldimethyl n-tetradecylammonium chloride or bromide, benzyl n-hexadecyldimethylammonium chloride or bromide or benzyl-dimethyl-n-octadecylammonium chloride or bromide, N-(n-decyl)-pyridinium chloride or bromide, N-(n-dodecyl)pyridinium chloride or bromide, N-(n-tetradecyl)pyridinium chloride or bromide, N-(n-hexa-decyl)pyridinium chloride or bromide (cetylpyridinium chloride or

bromide), or N-(n-octadecyl)pyridinium chloride or bromide, or an anionic surfactant of the formula IB, in particular sodium or potassium n-dodecyl (lauryl) sulfate, sodium or potassium n-tetradecyl (myristyl) sodium or potassium n-hexadecyl (cetyl) sulfate or sodium or potassium n-octadecyl (stearyl) sulfate, sodium or potassium n-dodecyloxyethyl sulfate, sodium or potassium n-tetradecyloxyethyl sulfate, sodium or potassium n-hexadexyloxyethyl sulfate or sodium or potassium n-octadecyloxyethyl sulfate, or an anionic surfactant of the formula IC, in particular sodium or potassium 2,2-dimethyl-3-palmitoyloxypropyl hydrogen phosphate, sodium or potassium 1-palmitoyllysophosphatidyl glycerol, sodium or potassium 1-palmitoyllysophosphatidylserine, and a lipid of the formula IC', wherein R_1 and R_2 are acyloxy, e.g. lauroyloxy, myristoyloxy, palmitoyloxy or stearoyloxy, R_3 is hydrogen and R_4 is 2-trimethylammonioethyl, e.g. a natural cephalin such as egg cephalin or cephalin or cephalin obtained from soybeans, or 2-aminoethyl, e.g. a natural lecithin such as egg lecithin or lecithin obtained from soybeans.

10. A process according to any one of claims 1 to 9, which comprises dispersing a homogeneous mixture of a surfactant and a lipid according to claim 9, and a pharmaceutical drug.

11. A process according to any one of claims 1 to 10, which comprises dispersing a homogeneous mixture of an anionic surfactant of the formula IB, egg lecithin and a muramyl peptide.

12. A process according to any one of claims 1 to 10, which comprises dispersing a homogeneous mixture of a cationic surfactant of the formula IA, soybean lecithin and a muramyl peptide.

13. A process according to claim 12, which comprises dispersing a homogeneous mixture of n-hexadecylpyridinium chloride, soybean lecithin and N-acetylmuramyl-L-alanyl-2-(1',2'-dipalmitoyl-sn-glycero-3'-phosphoryl)ethylamide.

14. A delivery system based on liposomes for encapsulated N-acetyl-muramyl-L-alanyl-D-isoglutamyl-L-alanyl-2-(1',2'-dipalmitoyl-sn-glycero-3'-phosphoro)ethylamide, prepared by the process as claimed in claim 1.

15. A pharmaceutical composition containing a delivery system based on liposomes for encapsulated drugs as claimed in claim 14, in combination with pharmaceutically acceptable adjuvants.

16. A delivery system according to claim 14 for use in the treatment of humans or animals.

17. A pharmaceutical composition according to claim 14 for use in the treatment of humans or animals.

18. A method of treating diseases in humans or animals, which comprises the use of a delivery system as claimed in claim 14.

DATED this 27th day of July 1983.

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